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Effects of non enzymatic antioxidants on serum total proteins and its fractions in Magra rams in arid region of Rajasthan

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Abstract
The objective of this study was to determine the effect of combination of vitamin E with selenium (Se) and vitamin C injections on serum total protein and its fractions in Magra rams. Twenty one Magra rams were randomly divided in three groups. The 1st group (n=7) was administered injection of normal saline (without antioxidant administration) 1ml SC once in a week as a placebo and was considered as a control (C). The rams of group 2nd (n=7) were given injections of selenium and vitamin E (1.5 mg sodium selenite and tocopherol 50mg/ml) 1ml SC per animal once in a week and considered as group VES. The rams of group 3rd (n=7) were given vitamin C (250mg sodium ascorbate/ml), 8 ml SC per animal on alternate days and considered as group VC. All the rams received the treatments for 45 days. Blood was collected twice in a week one month post-treatment for 5 consecutive weeks and serum was analyzed for serum total proteins and its fractions like albumin and globulin. Statistical analysis revealed significant (P<0.05) increase in concentration of serum total protein, albumin and globulin whereas A/G ratio remained unaffected by the treatment. The results of this experiment confirm a clear positive effect of parenteral administration of vitamin E combined with Se and vitamin C on serum total proteins and its fractions in rams.

Keywords: Vitamin E, selenium, vitamin C, rams, serum proteins, albumin, globulin

1. Introduction
Oxidative stress is an imbalance between the generation of reactive oxygen species and their neutralization by antioxidants, causes cellular damage (Trevisan *et al*., 2001; Paltrinieri, 2013) [52, 39] and degenerative diseases (McCord, 2000) [36] with ultimately reduction in reproductive potential. Reactive oxygen metabolites are involved in numerous signalling pathways that participate mainly in the management of anabolic and catabolic processes (Dröge, 2002) [18]. Antioxidants play an important role in protecting against the deleterious effects of oxidants (Castillo *et al*., 2003; Aytekin *et al*., 2010; Aytekin *et al*., 2011) [16, 10, 11]. Vitamin E and C are common and readily-available (Tugiyanti *et al*., 2014) chain breaking antioxidants which react with lipid radicals and convert them into more stable products (Anitra *et al*., 2000) [7]. Vitamin E and Se both can affect different biological processes like metabolism (Awadeh *et al*., 1998) [9], immunity (Hernken *et al*., 1998) [25], protection against oxidative stress (Bernabucci *et al*., 2002) [14] and growth as well as animal health (Underwood, 1977; McDowell, 2003; Surai, 2006) [54, 37, 49]. Vitamin C has numerous biological functions such as antioxidant (Weiss, 2006) that prevents the oxidation of protein (Seifi *et al*., 2010; Abdel-Monem *et al*., 2013) [46, 3] and immunity (Sahinduran and Albay, 2004; Kumar and Kataria, 2011) [45, 31].
Serum proteins act as carriers of lipids, hormones, vitamins and minerals in the circulatory system, and are involved in the regulation of cellular activity and the immune system (Anderson and Anderson 2002) [6]. Overall body protein status is routinely assessed through the levels of total proteins in serum or plasma, which include two major protein fractions: albumin and globulins. Albumin, due to its thiol groups acts as important antioxidant (Halliwell, 1988; Castillo *et al*., 2005; Roche *et al*., 2008) [21, 15, 41] and free-radical scavenger (Hankins, 2006) [23]. Serum albumin is a negative acute phase protein and its synthesis in the liver decreases in hepatic infection and injury (Thomus, 2000) [51]. Most of globulin proteins are synthesized in the liver which includes carrier proteins, enzymes and immunoglobulins.
Alterations in the concentration of serum protein and its fractions may occur not only under pathological,

but also under physiological conditions (Weaver *et al*. 2000; Janku *et al*. 2011) [55, 27] and may provide important diagnostic information to clinicians in confirming various abnormalities and diseases. However, studies dealing with changes in the serum protein pattern and in concentrations of protein fractions in rams with administration of non enzymatic antioxidants are still limited (Monzaly, 2000; Mahmoud *et al*., 2013) [38, 34]. Therefore, the aim of the present study was to evaluate the influence of non enzymatic antioxidants like vitamin E combined with Se and vitamin C on serum total proteins and its fractions in Magra rams.

2. Materials and Methods

The present study was undertaken in the department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, RAJUVAS and ICAR-CSWRI, Arid region Campus, Beechwal, Bikaner during year 2017-2018. Bikaner is located at 73˚18'E longitude and 28˚01'N latitude and at an altitude of 230 m above mean sea level. The area has arid environment and an annual rainfall ranging from 200-500 mm and annual ambient temperature ranges from 4˚C to 49˚C.

2.1 Housing and feeding management

The present study was conducted on twenty one Magra rams aged between 1.5-2.5 years having weight around 40 kg reared at CSWRI, ARC, Beechwal, Bikaner. Magra rams (n=21) were fed on the standard diet, formulated according to the requirement for mature ram suggested by Indian Council of Agricultural Research (ICAR, 2013). All the rams were maintained in identical managerial and feeding conditions throughout the study period and were provided pasture grazing for 7 hours per day throughout the period of study. All rams were maintained under proper hygienic conditions and had free access to water. During the experimental period all rams were housed in separate groups in well ventilated sheds made of asbestos roofing and open from sides. A general management program for deworming, disease prevention were followed during the experiment as prescribed by the health calendar of the institute to ensure that animals were remained in a healthy condition throughout the study.

2.2 Experimental design

Rams were randomly divided in three groups. The 1st group (n=7) was administered injection of normal saline (without antioxidant administration) 1ml SC once in a week as a placebo and was considered as a control (C). The rams of group 2nd (n=7) were given injections of selenium and vitamin E (1.5 mg sodium selenite and tocopherol 50mg/ml, Inj. Repronol, Cadila pharmaceuticals limited, Ahmedabad, India) 1ml SC per animal once in a week (Deori *et al.*, 2014) [17] and considered as group VES. The rams of group 3rd (n=7) were given vitamin C (250mg sodium ascorbete/ml, inj. Alpa--C, Alpa vet, Indore, India), 8 ml SC per animal on alternate days (Al-saab, 2015) [5] and considered as group VC. All the rams received the treatments for 45 days.

2.3 Collection of blood samples and analysis

Blood was collected twice in a week 1 month post-treatment for 5 weeks in sterilized 10ml tubes from treated and untreated rams by jugular veinepuncture. The serum was separated at 1000g for 20 minutes and stored at -20º C until analysis. Biochemical analysis of serum samples were done to estimate total protein, albumin (A), globulin (G), A/G ratio by VetTest biochemistry analyser (IDEXX Laboratories, US), as per the manufacturer's subscribed procedure. Serum globulin was estimated as a difference between total protein and albumin. A:G ratio was derived after dividing concentration of Albumin by concentration of Globulin.

2.4 Statistical analysis

Data obtained were analyzed statistically by three way analysis of variance using 3×5 factorial design and correlation were obtained using the SPSS computer programme (version 25.0), based on the standard procedures outlined by Snedecor and Cochran (1994) [48]. The mean values were compared by using Duncan's multiple range test (DMRT) described by Duncan (1955) [19].

3. Results

3.1 Serum total protein concentration

In present study, the overall mean serum protein concentration (g/dL) was 6.19±0.02, 7.35±0.04 and 7.1±0.05 in group C, VES and VC, respectively which was significantly (P<0.05) higher in VES group followed by VC than group C (Table 1). In current investigation, the overall mean serum protein concentration (g/dL) was 6.94±0.1, 7±0.09, 6.87±0.09, 6.78±0.09 and 6.8±0.09 during 1st, 2nd, 3rd, 4th and 5th week, respectively which differed significantly (P<0.05) between the weeks. The statistical analysis of data revealed significantly (P<0.05) higher overall mean serum protein concentration during the 1st and 2nd compared to 4th and 5th week. There was no significant difference between mean serum protein concentration during 1st, 2nd and 3rd weeks as well as between 3rd, 4th and 5th weeks. Analysis of variance revealed no significant interaction between treatment groups and weeks (Table 1).

3.2 Serum albumin concentration

In the present study, the overall mean serum albumin concentration (g/dL) was 2.53±0.01, 2.99±0.02 and 2.89±0.03 in group C, VES and VC, respectively which was significantly (P<0.05) higher in group VES followed by VC than group C (Table 2).

The overall mean serum albumin concentration (g/dL) was 2.86±0.04, 2.87±0.04, 2.8±0.04, 2.73±0.04 and 2.75±0.03 during 1st, 2nd, 3rd, 4th and 5th week, respectively which differed significantly (P<0.05) between the weeks. During 2nd week, overall mean serum albumin concentration was significantly (P<0.05) higher compared to rest of the weeks. During 1st and 3rd week, the overall mean serum albumin concentrations were significantly (P<0.05) higher than 4th week and differed non significantly with each other. The mean serum albumin concentration had no significant difference between 3rd and 5th week as well as between 4th and 5th week. Analysis of variance revealed significant (P<0.05) interaction between treatment groups and weeks (Table 2).

3.3 Serum globulin concentration

In the current experiment, the overall mean serum globulin concentration (g/dL) was 3.66±0.02, 4.36±0.03 and 4.11±0.04 in group C, VES and VC, respectively which was significantly (P<0.05) higher in group VES followed by VC than group C. In the present investigation, the overall mean serum globulin concentration (g/dL) was 4.04±0.06, 4.1±0.06, 4.04±0.06, 4.01±0.06 and 4.04±0.06 during 1st, 2nd, 3rd, 4th and 5th week, respectively which differed non significantly between the weeks. Analysis of variance revealed no

significant interaction between treatment groups and weeks (Table 3).

3.4 Serum albumin-globulin (A:G) ratio

In the current experiment, the overall mean serum albumin globulin ratio was 0.7±0.01, 0.69±0.01 and 0.71±0.01 in group C, VES and VC, respectively which differed non significantly between the treatment groups. The overall mean serum albumin globulin ratio was 0.72±0.01, 0.71±0.01, 0.7±0.01, 0.69±0.01 and 0.69±0.01 during 1st, 2nd, 3rd, 4th and 5th week, respectively which differed non significantly between the weeks. Analysis of variance revealed no significant interaction between treatment groups and weeks (Table 4).

Table 1: Effect of vitamin E+Se and vitamin C on total serum protein concentration (g/dL) in Magra rams (Mean±SE)

Groups	Serum protein concentration (g/dL) during weeks (n=10)					Over all
	1	2	3	4	5	
C	6.19 ±0.06	6.24±0.05	6.2±0.03	6.19±0.05	6.09±0.05	6.19 ± 0.022^{a}
VES	7.39±0.11	7.44±0.08	7.32±0.11	7.32±0.07	7.25±0.06	7.35 ± 0.039^{c}
VC	7.23±0.09	7.31±0.07	7.08±0.13	6.82±0.12	7.06±0.09	7.1 ± 0.048^{b}
Over all	6.94 ± 0.096^{B}	7 ± 0.092^{B}	6.87 ± 0.094^{AB}	6.78 ± 0.086^{A}	6.8 ± 0.088^{A}	

Means having different superscripts in a column (small letter) differ significantly ($P<0.05$)
Means having different superscripts in a row (capital letter) differ significantly ($P<0.05$)
n=Number of serum samples

Table 2: Effect of vitamin E+Se and vitamin C on serum albumin concentration (g/dL) in Magra rams (Mean±SE)

Groups	Serum albumin concentration (g/dL) during weeks (n=10)					Over all
	1	2	3	4	5	
C	2.57 ± 0.02^{aA}	2.52 ± 0.03^{aA}	2.53 ± 0.04^{aA}	2.51 ± 0.03^{aA}	2.51 ± 0.02^{aA}	2.53 ± 0.01^{a}
VES	3.02 ± 0.06^{bB}	3.07 ± 0.03^{bB}	2.99 ± 0.05^{cAB}	2.97 ± 0.04^{cAB}	2.87 ± 0.03^{bA}	2.99 ± 0.02^{c}
VC	2.99 ± 0.02^{bC}	3.01 ± 0.03^{bD}	2.87 ± 0.07^{bB}	2.69 ± 0.07^{bA}	2.86 ± 0.04^{bB}	2.89 ± 0.03^{b}
Over all	2.86 ± 0.039^{C}	2.87 ± 0.043^{D}	2.8 ± 0.042^{BC}	2.73 ± 0.041^{A}	2.75 ± 0.032^{AB}	

Means having different superscripts in a column (small letter) differ significantly ($P<0.05$)
Means having different superscripts in a row (capital letter) differ significantly ($P<0.05$)
Means carrying different superscripts (small and capital letter both) have significant interaction ($P<0.05$)
n=Number of serum samples

Table 3: Effect of Vitamin E+Se and vitamin C on serum globulin concentration (g/dL) in Magra rams (Mean±SE)

Groups	Serum globulin concentration (g/dL) during weeks (n=10)					Over all
	1	2	3	4	5	
C	3.62±0.06	3.73±0.05	3.67±0.05	3.69±0.06	3.58±0.05	3.66 ± 0.02^{a}
VES	4.37±0.07	4.37±0.07	4.34±0.07	4.36±0.07	4.38±0.06	4.36 ± 0.03^{c}
VC	4.12±0.09	4.2±0.07	4.09±0.09	3.98±0.09	4.14±0.08	4.11 ± 0.04^{b}
Over all	4.04±0.064	4.1±0.055	4.04±0.059	4.01±0.061	4.04±0.064	

Means having different superscripts in a column (small letter) differ significantly ($P<0.05$)
n=Number of serum sample

Table 4: Effect of vitamin E+Se and vitamin C on serum albumin-globulin (A:G) ratio in Magra rams (Mean±SE)

Groups	Serum albumin-globulin (A:G) ratio during weeks (n=10)					Over all
	1	2	3	4	5	
C	0.71±0.01	0.68±0.01	0.69±0.02	0.69±0.02	0.70±0.01	0.7±0.01
VES	0.7±0.02	0.71±0.01	0.69±0.01	0.68±0.02	0.66±0.01	0.69±0.01
VC	0.73±0.02	0.72±0.02	0.7±0.02	0.68±0.02	0.7±0.02	0.71±0.01
Over all	0.72±0.009	0.71±0.009	0.7±0.009	0.69±0.011	0.69±0.009	

Means having different superscript differ significantly ($P<0.05$)
n=Number of serum samples

4. Discussion

4.1 Serum total protein

In the present study, the mean serum protein concentration was significantly ($P<0.05$) higher in VES group followed by VC than group C. The statistical analysis of data revealed significant ($P<0.05$) difference of mean serum protein concentration between the weeks whereas no significant interaction was observed between treatment groups and weeks. Similar findings were recorded by Mahmoud *et al*., (2013; 2014) [34, 15] in Ossimi rams and revealed significant increase in serum total protein concentration. In accordance with the present study, feeding additional antioxidants (vitamin E/Se) had significantly improved levels of total serum protein in Baladi ewes (El-Shahat and Abdel Monem, 2011) [20] and buffaloes (Helal *et al*., 2009) [24]. In agreement with the present study, higher levels of total protein was reported following Se and vitamin E supplementation in pregnant ewes and lambs (Avci *et al*., 2000; Pisek *et al*., 2008) [8, 40]. Our findings are in agreement with reports of Sahin *et al*., (2002) [42] and Sahin *et al*., (2003) [43] who evaluated that dietary ascorbic acid supplementation increased serum total protein in quails and broilers, respectively. In accordance with the present experiment, the ameliorative effect of ascorbic acid supplementation was studied by Abd-Allah and Zanouny, (2014) [1] who noted significantly higher total protein concentration ram lambs.

In contrast to the present study, effect of vitamin E and Se on serum total protein was found to be non significant in buffalo

calves (Shinde *et al*., 2009) [47] and sheep (Hamam and Abou-Zeina, 2007) [22]. Also, supplementation of Se had no effects on the concentrations of serum total protein in lambs (Kumar *et al*., 2008; Kumar *et al*., 2009) [32] and Raieni goats (Ziaei, 2015) [58]. A few previous studies found that, supplementation of ascorbic acid had no significant effect on mean value of serum total protein in ewes (Babe, 2011) [12] and Swamp buffaloes (Konwar *et al*., 2017) [30].

The exact mechanism of higher serum total protein in supplemented animals was not clear but increased concentration of γ globulin could be considered as cause.

4.2 Serum albumin

In the present study, mean serum albumin concentration was significantly (P<0.05) higher in group VES followed by VC than group C. The overall mean serum albumin concentration was significantly (P<0.05) different between the weeks and interaction between treatment groups and weeks also revealed significantly (P<0.05) different. In agreement with present results, significant increase in serum albumen value was reported following vitamin E and Se supplementation in pregnant ewes, lambs (Avci *et al*., 2000; Pisek *et al*., 2008) [8, 40] and rams (Mahmoud *et al*., 2013) [34]. These findings come in agreement with previous reports that showed significantly higher value of blood albumen concentration treated with vitamin C in buffalo bulls (Youssef *et al*., 2013) [57] and ewes during post lambing (Monzaly, 2000) [38].

In contrast to the present findings, Hamam and Abou-Zeina (2007) [22] didn't observed significant importance of the effect of vitamin E and Se on the serum concentrations of albumin in sheep. Similarly, supplementation of Se had no effect on the concentrations of serum albumen concentration in lambs (Kumar *et al*., 2008; 2009) [32] and in Raieni goats (Ziaei, 2015) [58]. Kobeisy (1994) [29] found that there was no significant effect of dietary vitamin C on serum protein concentrations in buffalo and Jersey growing calves. The oral administration of vitamin C for treating Friesian cows did not affect concentrations of albumin (Abu El-Hamad *et al*., 2007) [4]. This result may be due to improvement of protein anabolism, decrease of protein catabolism. Some authors suggested that the increase of blood protein concentrations of vitamin C treated animals may be related to the important role of vitamin C in amino acids metabolism and protein synthesis (Abdel-Wahab *et al*., 1975) [2].

4.3 Serum globulin

In the current study, the mean serum globulin concentration was significantly (P<0.05) higher in group VES followed by VC than group C whereas there was non significant difference between the weeks. Analysis of variance revealed no significant interaction between treatment groups and weeks. In accordance with the present study, significantly increased serum globulin concentration was observed in Ossimi rams (Mahmoud *et al*., 2013 and 2014) [34, 35] following treatment with vitamin E plus Se and Baladi sheep (El-Shahat and Abd El-Monem, 2011) [20]. The present results come in accordance with earlier study of Ibrahim, (2017) [26] who found significantly higher concentrations of serum globulin after parenteral administration of vitamin E plus Se (@ 1.0 ml/head) compared to untreated and lower dose (@ 0.5 ml/head) in Ossimi ram lambs. The present data come in accordance with previous work of Monzaly (2000) [38], who noted significant increase in globulin concentration in blood during post lambing ewes treated with vitamin C. In agreement with the present finding, Abu El-Hamad *et al*, (2007) [4] observed that concentrations of globulin increased significantly by increasing daily vitamin C dose up to 1 g/cow/ day concomitant with improve in milk production of cows.

In contrast to the presented results, Shinde *et al*. (2009) [47] showed no effect of vitamin E plus Se supplement on serum globulin in buffalo calves. Shushma et al., (2015) found that the level of dietary Se supplementation didn't influenced serum globulin concentration. Similarly, Kumar *et al*., (2009) [32] observed that supplementation of Se at 0.15 ppm level either from organic or inorganic source had no effect on the values of serum globulin concentration. The dietary ascorbic acid supplementation did not significantly affect the serum globulin in male Meriz kids (Barwary *et al*., 2011) [13] whereas significantly decreased in calves (Kim *et al*., 2012) [28].

4.4 Serum albumin-globulin (A:G) ratio

In the current experiment, the mean serum albumin-globulin ratio differed non significantly between the treatment groups and weeks as well as interaction between treatment groups and weeks also differed non significantly. Ratio obtained from present study is in agreement with Mahmoud *et al*., (2013; 2014) [34, 35] who found similar results by pareteral administration of vitamin E and Se in Ossimi rams. The present results are in accordance with earlier study of Kumar *et al*., (2008; 2009) [32] who noted that organic or inorganic supplementation of Se ha no effects on the concentrations of serum A-G ratio in lambs. Present results stand with findings of Seifi *et al*., (2010) [46] who observed non significant differences for serum A:G ratio between ascorbic acid treated and untreated neonatal dairy calves. Similarly, intramuscular administration of ascorbic acid at the rate of 6ml/head/week didn't affect A-G ratio in buffalo bulls (Youseef *et al*., 2013) [57].

5. Conclusion

In conclusion, our results demonstrated a clear positive effect of parenteral administration of vitamin E combined with Se and vitamin C on serum total proteins and its fractions in rams. It seems that *in vivo* production of vitamin C may not be sufficient for optimum biological processes. These non enzymatic antioxidants mitigate the oxidative stress and ultimately beneficial for maintaining overall health of the rams.

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